Example 10: The in Vivo Effect on Proliferation of Combining an MDM2 Inhibitor with a MEK Inhibitor and a BCL-2/-XL Inhibitor in a TP53 Wild-Type Model of Colorectal Cancer

Generation of Tumor-Bearing Mice and Treatment

[0257] All animal experiments were done in strict adherence to the Swiss law for animal protection. Female Crl: NU(NCr)-Foxn1nu mice were purchased from Charles River Laboratories International Inc (Germany) and kept in a pathogen-controlled environment. Subcutaneous tumors of HCT-116 (KRAS mutant, PIK3CA mutant, p53 wild-type) were induced by concentrating 3 million cells in 100 μl of PBS and injecting them in the right flank of nude mice. The mice were randomly grouped, and treatment was started when the tumor size reached 50 to 250 mm3. Each cohort included 8 mice. Tumor sizes were monitored three times weekly, and volumes were calculated with the following formula: (mm3)=length×width2×0.5.

[0258] The MDM2 inhibitor (S)-1-(4-Chloro-phenyl)-7-isopropoxy-6-methoxy-2-(4-{methyl-[4-(4-methyl-3-oxo-piperazin-1-yl)-trans-cyclohexylmethyl]-amino}-phenyl)-1,4-dihydro-2H-isoquinolin-3-one (COMPOUND A), the MEK inhibitor trametinib (COMPOUND B), and the BCL-2/-XL inhibitor ABT-263 (COMPOUND C) in powder form were stored at +4° C. COMPOUND A was dissolved in 0.5% hydroxypropyl methylcellulose, COMPOUND B was dissolved in 1% carboxymethylcellulose containing 0.5% Tween-80% in distilled water (pH7.6-8.0), and COMPOUND C was dissolved in Microemulsion pre-concentrate 5. All drugs were dosed orally using 5-10 ml/kg. COMPOUND A was administered three times a week (3qw) at 100 mg/kg. COMPOUND B and COMPOUND C were administered daily (q24 h) at 0.3 and 100 mg/kg, respectively. The combination dosing schedule and dosage were the same as the single reagents.

[0259] Six treatment cohorts (G1-G6) were tested:

[0260] G1: vehicle (DMSO)

[0261] G2: COMPOUND C

[0262] G3: COMPOUND A=>after 9 days treatment COMPOUND C was added

[0263] G4: COMPOUND B=>after 9 days treatment COMPOUND C was added

[0264] G5: COMPOUND A+COMPOUND B=>after 9 days treatment COMPOUND C was added

[0265] G6: COMPOUND A+COMPOUND B+COMPOUND C

Statistical Analysis

[0266] For each tumor at each time-point the size was normalized to the size before the start of the treatment to obtain the "% Change tumor volume" (FIG. **17-18**, y-axis). For FIG. **17** at each time point the mean size of all tumors per cohort was calculated, and the error of the size using the standard error of the mean (SEM). For FIG. **18** p-values were calculated using a one-tailed t test.

Results

[0267] In a xenograft model of HCT-116 cells the combination treatment of COMPOUND A and trametinib (G5) was significantly better (stable disease) when compared to each of the single agent treatments (G3-G4 showing progressive disease), and the triple combination of COMPOUND A, trametinib, and ABT-263 (G6) led to marked tumor regression and had a significantly better response compared to G5 (FIG. **17** and FIG. **18**A). FIG. **17** shows summarized survival curves, and FIG. **18** shows waterfall plots at day 9 and day 19 after start of the treatments. Sequential addition of ABT-263 after 9 days to single agent COMPOUND A had no additional benefit, while it stopped tumor progression when added to single agent trametinib. ABT-263 led to marked tumor regressions when added to the combination of COMPOUND A and trametinib, and at day 19 the responses of concomitant and sequential treatments were indistinguishable (FIG. **18**B).

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